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Dated 10 June 2004

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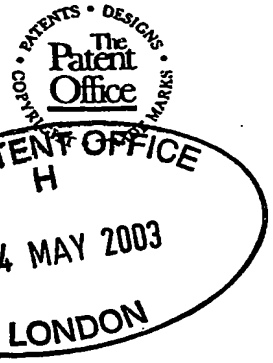

1/77
15MAY03 E807275-1 D02114
P01/7700 0.00-0311081.4

14 MAY 2003

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
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1. Your reference — 143884

2. Patent application number *(The Patent Office will fill in this part)* — 0311081.4

3. Full name, address and postcode of the or of each applicant *(underline all surnames)* — BTG INTERNATIONAL LIMITED

   10 Fleet Place
   Limeburner Lane
   London
   EC4M 7SB
   GB

   Patents ADP number *(if you know it)* — 08084006001

   If the applicant is a corporate body, give the country/state of its incorporation — GB

4. Title of the invention — Treatment of Neurodegenerative Conditions

5. Name of your agent *(if you have one)* — Dolan, Anthony

   "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

   BTG INTERNATIONAL LIMITED
   10 Fleet Place
   Limeburner Lane
   London
   EC4M 7SB
   GB

   Patents ADP number *(if you know it)* — 08084006001 GB

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
   a) any applicant named in part 3 is not an inventor, or
   b) there is an inventor who is not named as an applicant, or
   c) any named applicant is a corporate body.
   See note (d)) — Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	10
Claim(s)	3
Abstract	-
Drawing(s)	5

10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	1
Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Signature CD England Date

ENGLAND, Christopher David 14/05/03

12. Name and daytime telephone number of person to contact in the United Kingdom

Steven Bayliss – 020 7575 1584

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TREATMENT OF NEURODEGENERATIVE CONDITIONS.

The present invention relates to a method for treating neurodegenerative conditions, particularly those in which transforming growth factor β (TGF-β) is implicated, particularly TGF-β1. More particularly the present invention provides treatment for conditions such as multiple sclerosis and the degenerative sequelae associated with head trauma, stroke and intracranial bleeds. Further provided are novel use of known and novel compounds comprising unsaturated fatty acid moieties for the manufacture of medicaments capable of effectively treating such conditions, more particularly being capable of achieving previously unattained levels of success with regard to recovery of neurological function.

It is well reported in the literature that essential fatty acids of the n-3 and n-6 unsaturation pattern have beneficial effect in a wide variety of human physiological disorders. Harbige (1998) Proc. Nut. Soc. 57, 555-562 reviews the supplementation with n-3 and n-6 acids in autoimmune disease states, particularly noting evidence of benefit of γ-linolenic (GLA) and/or linoleic acid (LA) rich oils, such as borage oil, in reducing clinically important signs and symptoms of rheumatoid arthritis.

Two studies on multiple sclerosis patients are noted that indicate that relapse and severity of the disease might be reduced by treatment with oils containing n-6 acid moieties(Miller et al (1973) and Bates et al (1978)), with a further study failing to confirm this effect (Paty et al (1978).

These papers report that supplementation of human patients with about 20g/day of linoleic acid affected duration and severity of relapses of multiple sclerosis such that relapses were less frequent, less severe and of shorter duration than controls. Bates noted that a mixture of linoleic acid and γ-linolenic acid had been suggested back in 1957 to be possibly more efficacious in treating inflammation and autoimmune diseases and set out to investigate this in the trial. However, it was found that where this combination was tried, at 3g oil per day (Naudicelle Evening Primrose oil) patients who had relapses became more ill on the trial oil than on the control.

Meta analysis of these linoleic acid studies by others (Dworkin et al (1984)) demonstrated reduced relapse rate and severity with a decrease in the degree of long term progression of the disease in patients with mild multiple sclerosis. Open studies of patients with multiple sclerosis suggest that low fat diet and/or manipulation of dietary n-3 and n-6 fatty acids may be beneficial (Swank & Grimsgaard (1988); Harbige et al (1990).

Experimental autoimmune encephalomyelitis (EAE) is the most frequently used animal model for immune mediated effects of MS. Studies in the guinea-pig have shown that linoleic acid partially suppresses the incidence and severity of EAE (Meade et al (1978) whilst high levels of linoleic rich oil containing low levels of γ-linolenic acid (linoleic acid:γ-linolenic acid 7:1) partially suppressed the incidence and severity of EAE in rat (Mertin & Stackpoole, 1978). Using γ-linolenic acid-rich oils from fungal or plant sources, complete protection was demonstrated in both rats and mice (Harbige et al (1995), 1997b). These investigations demonstrated disease modifying effects of linoleic acid and γ-linolenic acid on clinical and histopathological manifestations of EAE. Depending on dose, γ-linolenic acid was fully protective in acute rat EAE whereas linoleic acid had dose-dependent action on the clinical severity but did not abolish it.

Despite these experimental findings, it is recognised that the human disease, multiple sclerosis, is highly complex and can be conversely exacerbated and ameliorated by the activity of T-cells and other immune response factors. The n-6 fatty acids promote autoimmune and inflammatory disease based upon results obtained with linoleic acid only. TGF-β and PGE_2 production has been shown to be increased non-specifically in γ-linolenic acid fed mice *ex vivo*; whilst TGF-β has been reported to protect in acute and relapsing EAE ((Racke et al (1993); Santambrogio et al (1993)) and PG inhibitors such as indomethacin augment the disease (Ovadia & Paterson (1982)).

During natural recovery phase from EAE, TGF-β-secreting T-cells inhibit EAE effector cells, TGF-β is expressed in the CNS and, in oral-tolerance-induced

protection in EAE, TGF-β and PGE_2 are expressed in the brain (Karpus & Swanborg (1991); Khoury et al (1992)). Harbige ((1998) concluded that dietary γ-linolenic acid effects on EAE are mediated through Th_3-like mechanisms involving TGF-β and possibly through superoxide dismutase antioxidant activity.

Borage oil (23% γ-linolenic acid and 62% linoleic acid per 100% fatty acid content) has been shown to significantly reduce clinically important signs and symptoms of autoimmune disease associated with active rheumatoid arthritis (Leventhal et al (1993)). Borage oil and fungal oil (see Figure 1) have been shown to be effective in the EAE animal model of multiple sclerosis, whilst never having been show to be significantly effective in the human disease. In spite of the use of borage oil and other GLA/LA containing oils such as evening primrose oil by multiple sclerosis sufferers over the past 30 years or so, the vast majority of patients fail to recover from the disease or show significant improvement, with the underlying disease continuing to progress.

Other more dramatic immunosuppressant treatments, including T cell depleters and modulators such as cyclophosphamide, are also shown to be effective in the EAE model, but where these are employed in the human multiple sclerosis disease symptoms improve, but the underlying disease continues to progress. The 'gold standard' treatment for MS remains interferon, such as with β-Avonex ® and other interferon preparations. This gold standard treatment only addresses needs of some, eg 30%, of the patients and even in these symptom improvement is restricted

The present inventors have now surprisingly determined that with compliance to a 'high dose' treatment containing both γ-linolenic acid and linoleic acid remarkable levels of improvement in almost all symptoms of MS can be achieved, way surpassing that provided by the present gold standard treatment. Such success is particularly surprising in the light of the prior use of such preparations without such significant success..

In a first aspect of the present invention there is provided a method of treating a patient in need of therapy for a neurodegenerative disease comprising administering to that patient a therapeutically effective dose of an oil containing both γ-linolenic

acid and linoleic acid residues as triglyceride ester, the ratio of γ-linolenic acid to linoleic acid residues being from 1:2 to 2:1 and the total of these being at least 30% of the esterified fatty acid content of the oil, wherein the oil is administered at a dose sufficient to maintain or elevate TGF-β levels in the patient to therapeutic levels.

Most preferably, in addition to the γ-linolenic acid and linoleic acid fatty acid residues, the oil includes oleic acid residues. By residue is meant the moiety that remains after the fatty acid carboxyl group esterifies to one of the hydroxy groups of the glycerol molecule.

Most preferably the oil administered is an oil source from Borage oil or a fungal oil eg. eg from Mucor,

Typical Borage oil and fungal oil compositions are illustrated in Table 1 wherein 18:2n-6 and 18:3n-6 represent linoleic and γ-linolenic acid residue by percent respectively.

Typically Borage oils contain from 20 to 25% γ-linolenic acid residues as percentage of fatty acid residues in the oil and from 35 to 40% linoleic acid residues.

For treatment regimes where high amounts of oil are administered it is recommended that the amount of potentially toxic fatty acids, such as erucic acid, are as low as possible, preferably lower than 5% of fatty acid residues, more preferably less than 3% and more preferably less than 2%.

EXAMPLES.

Twenty-eight active relapsing-remitting (two relapses in the preceding 18 months) multiple sclerosis patients (ages ranging from 18 t0 65 yrs) were entered into a double-blind placebo controlled trial to investigate the effects of encapsulated borage oil on clinical activity and laboratory parameters over 18 months.

Patients were recruited from neurology out-patient clinics at two inner city hospitals; hospital informed consent was obtained on first (baseline) visit. Exclusion criteria include any form of steroid or immunosuppressive drug treatment, pregnancy, hyperlipidemia, regular use of aspirin or related drugs and vitamin or fatty acid

supplementation within the previous three months. Patients were randomly allocated by the Pharmacy Department to one of three groups each containing 12 patients:

- One clinical group (n=12) to receive placebo (5 g of Polyethylene Glycol 400)
- Second clinical group (n=12) to receive low-dose (5 g) refined *Borage officinalis*
- Third clinical group (n=12) to receive high-dose (15 g) refined *Borage officinalis*

Supplementation was in the form of one gram oil capsules daily (5/day for low dose, 15/day high dose) for 18 months duration. *Borage officinalis* oil and omega-6 polyunsaturated fatty acids are food ingredients that are generally recognised as safe for human consumption (GRAS). There are no classification or labelling requirements under EC regulations. Clinical assessment included: Extended Disability Scale Scores (EDSS) and clinical relapse record. Venous blood (50 mls) was obtained for laboratory studies on the 1st, 3rd, 6th, 12th, 15th, and 18th month of supplementation.

The following biochemical and immunological parameters were investigated on each visit for comparison with pre-treatment data and between group data:

- Stimulated and unstimulated *ex vivo* peripheral blood mononuclear cell cytokine production: TGF-β1, IFN-γ, TNF-α, IL-1β, IL-6 and IFN-β, which are implicated in the pathogenesis of MS. Cytokine and related gene expression.
- Soluble adhesion molecules in serum particularly ICAM-1 and VCAM-1
- Peripheral blood mononuclear cell membrane fatty acids and plasma phospholipid fatty acid composition.

Results are shown in Tables 1 and 2 and Figures 1 to 5.

Only patients meeting all the following criteria were included in the trial: (a) able to provide informed consent prior to treatment, with the full understanding that consent may be withdrawn at any time without prejudice; (b) male or female out-patients aged 18 to 60 years inclusive; (c) have confirmed diagnosis of clinically definite relapsing MS; (d) have had at least three documented clinical relapses in the past two years; (e)

have a baseline Expanded Disability Scoring Scale (EDSS) score of 0.0-5.5 inclusive, provided they have well documented exacerbations; and (f) healthy, apart from the MS-related symptoms, as confirmed by the medical history, physical examination and clinical chemistry, urine and haematological tests. The primary outcome parameter was the number of clinical relapses between baseline (Month 0) and the end of treatment (Month 18). Secondary outcome parameters included: the time to first clinical relapse; severity of relapses, as assessed by EDSS score and the use of steroid treatment; and changes in EDSS at Month 3, 6, 9, 12, and 18 compared to baseline and defined as at least 1.0 point increase in the EDSS that is sustained for 3 months or at least 1.5 point increase on the EDSS from the baseline EDSS that is sustained for 3 months. As this trial did not receive external funding, it was not possible for financial reasons to evaluate MS diseases activity with magnetic resonance imaging. 1 of 3

Eleven patients were in the placebo group, seven patients had been taking low-dose Borage oil, and ten patients had been taking high-dose Borage oil. The study drug was well-tolerated, and there were no serious adverse events during the 18-month trial.

Two patients had developed diarrhoea, both of whom were later confirmed to have been taking high-dose Borage oil. The diarrhoea was mild in one patient, but was moderately severe in the second patient, who later discontinued the study drug. The code was not broken and the diarrhoea had stopped after the discontinuation of the drug, but reappeared upon re-challenge. Therefore, this patient was withdrawn from the trial. The remaining patients who were treated with high-dose Borage oil showed excellent clinical improvement on all primary and secondary outcome criteria. For example, their mean EDSS score after 6 months of treatment had improved from baseline EDSS (Figure 1). More importantly, the mean number of clinical relapses had significantly reduced after 6 months of treatment when compared to the number of relapses in the placebo group (Figure 2). In contrast, patients who had been receiving low-dose Borage oil did not show any clinical improvement when compared to the placebo group. In addition to its beneficial effect on MS disease activity, high does Borage oil provided some symptomatic relief of muscle spasticity

(stiffness) and painful sensory symptoms, and also improved cognitive functions.

The following are two brief case histories to illustrate the therapeutic benefits of Borage oil.

Patient 1 (Treatment):

The first patient was a 48-year old woman who had had a clinically active, relapsing remitting MS for 9 years. She had originally worked as a full-time administrator at the local Health Authority, but she was unable to perform her duties because of her severe MS. Therefore, she later worked as a part-time secretary, but still had difficulties in mobilization because of muscles stiffness and sensory disturbances. She was also experiencing severe clinical relapses at an average of one relapse every nine months. Most of these relapses had resulted in hospital admissions for steroid therapy. In view of her active MS, she was recruited into the Borage oil trial. There were no adverse events relating to the study, and after taking the medication for four months, she experienced good improvement in her walking and sensory symptoms.

About nine months after therapy, she was well enough to start full-time employment. In addition, she remained relapse-free for the 18-month duration of the clinical trial. Following the conclusion of the trial, the treatment code revealed that she was taking high-dose Borage oil.

Patient 2 (Control):

The second case was a 46-year old woman who also had a clinically active relapsing remitting MS for 8 years. She had originally worked as a shop assistant, but became unemployed after MS was diagnosed.

Her symptoms included difficulty with mobilisation and painful sensory symptoms in both legs. She had experienced three clinical relapses in the two years preceding the clinical trial, and had been admitted to hospital twice for steroid therapy. Consequently, she was recruited into the Borage oil trial, but her walking continued to deteriorate. Six months into the trial, she need to use a walking stick and also received treatment with Baclofen to reduce low limb spasticity. Approximately ten months after starting the Borage oil trial, she was admitted to hospital because of

sever clinical relapse, which was treated with steroids. She later developed bladder disturbances and began to use a wheelchair for long journeys. The treatment code was broken after the conclusion of the 18-month trial, and she was found to have been taking placebo. Since then, she started using a walking frame for journeys exceeding 50 yards.

TABLE 1

Compositional (% Total FAs) Characteristics of Various Oils and their Protective Effects in EAE

Treatment	18:2n-6	18:3n-6	18:2n-6/18:3n-6	18:1n-9	INCIDENCE OF EAE
FGO	17	20	0.6	35	0/10
BOO	37	24	1.5	15	3/10
EPO	71	9.4	7.5	9	7/10
SAF	66	-	-	17	9/10
Controls	-	-	-	-	9/10

FGO, Fungal Oil; BOO, Borage Oil; EPO, Evening Primrose Oil, SAF, Safflower Oil.

TABLE 2

Treatment Groups- Borage oil-MS trial

		Female	Male	Mean Relapse Rate (in past two years)	Mean Base EDSS	Number
Group	Placebo	7	4	2.6	3.9	11
	Low Dose	5	2	2.9	3.5	7
	High Dose	8	2	3.4	2.8	10
Total		20	8	2.9	3.4	28

CLAIMS.

1. Use of an oil containing both γ-linolenic acid and linoleic acid residues as triglyceride ester, the ratio of γ-linolenic acid to linoleic acid residues being from 1:2 to 2:1 and the total of these being at least 30% of the esterified fatty acid content of the oil for the manufacture of a medicament for treatment of a disease benefiting from increased TGF-β levels.

2. Use of an oil containing both γ-linolenic acid and linoleic acid residues as triglyceride ester, the ratio of γ-linolenic acid to linoleic acid residues being from 1:2 to 2:1 and the total of these being at least 30% of the esterified fatty acid content of the oil for the manufacture of a medicament for treatment of neurodegeneration.

3. Use of an oil containing both γ-linolenic acid and linoleic acid residues as triglyceride ester, the ratio of γ-linolenic acid to linoleic acid residues being from 1:2 to 2:1 and the total of these being at least 30% of the esterified fatty acid content of the oil, for the manufacture of a medicament for treatment of multiple sclerosis.

4. Use as claimed in any one of the preceding claims characterised in that the oil comprises at least 20% of the esterified fatty acid content as γ-linolenic acid residues

5. Use as claimed in any one of the preceding claims characterised in that the oil is a Borage oil.

6. A method of treating a patient in need of therapy for a neurodegenerative disease comprising administering to that patient a therapeutically effective dose of an oil containing both γ-linolenic acid and linoleic acid residues as triglyceride ester, the ratio of γ-linolenic acid to linoleic acid residues being from 1:2 to 2:1 and the total of these being at least 30% of the esterified fatty acid content of the oil, wherein the oil

is administered at a dose sufficient to maintain or elevate TGF-β levels in the patient to therapeutic levels.

7. A method as claimed in Claim 1 characterised in that the neurodegenerative disease is multiple sclerosis.

8. A method as claimed in Claim 1 characterised in that the effective dose comprises at least 2 grams equivalent γ-linolenic acid residues per day.

9. A method as claimed in Claim 1 characterised in that the effective dose comprises at least 3 grams equivalent γ-linolenic acid residues per day.

10. A method as claimed in Claim 1 characterised in that the effective dose comprises 10 grams or more of borage oil per day.

11. A method as claimed in Claim 1 characterised in that the effective dose comprises 15 grams or more of borage oil per day.

12. A method as claimed in Claim 1 characterised in that the oil has erucic acid content less than 5% of the fatty acid residues in the oil.

13. A method as claimed in Claim 1 characterised in that the effective dose is administered orally, daily, for at least 4 months.

14. A method as claimed in Claim 8 characterised in that the effective dose is administered orally, daily, for at least 6 months.

15. A method as claimed in Claim 1 characterised in that the dosage is sufficient to relieve muscle spasticity and/or pain.

16. A method as claimed in Claim 1 characterised in that the dosage is sufficient to improve cognitive function.

17. A method as claimed in Claim 1 characterised in that the dosage is sufficient to eliminate relapses..

18. A method as claimed in Claim 1 characterised in that the dosage is sufficient to improve the patients EDSS score by at least 1 unit over a period of 1 years treatment.

19. A method as claimed in Claim 1 characterised in that the dosage is sufficient to restore EDSS of a patient with EDSS above 2.5 to below 2 over a period of 1 years treatment.

FIGURE 1 **Peripheral Blood Mononuclear Cell Cytokine Production in Placebo and Oil Treated Multiple Sclerosis Patients at 18 Months**

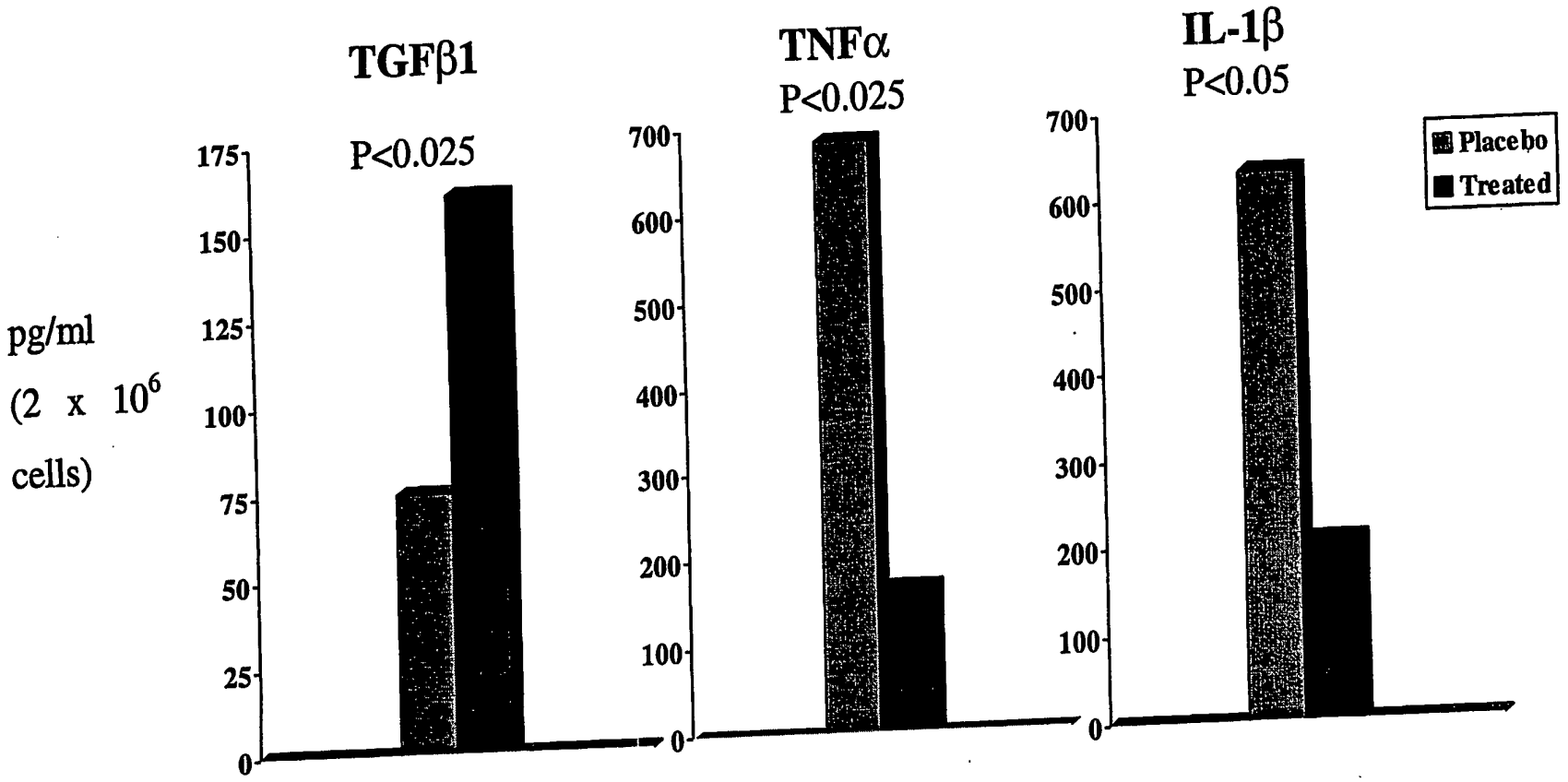


FIGURE2

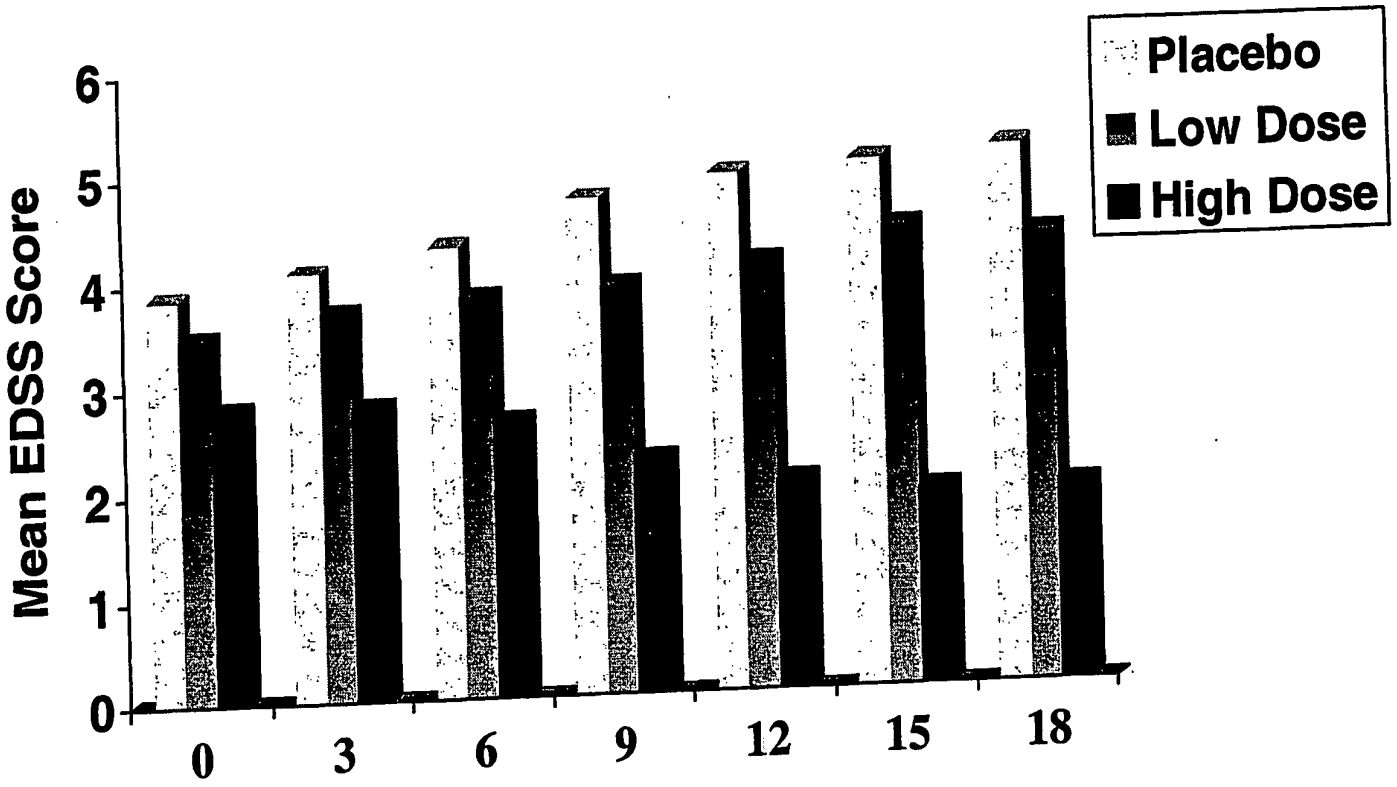
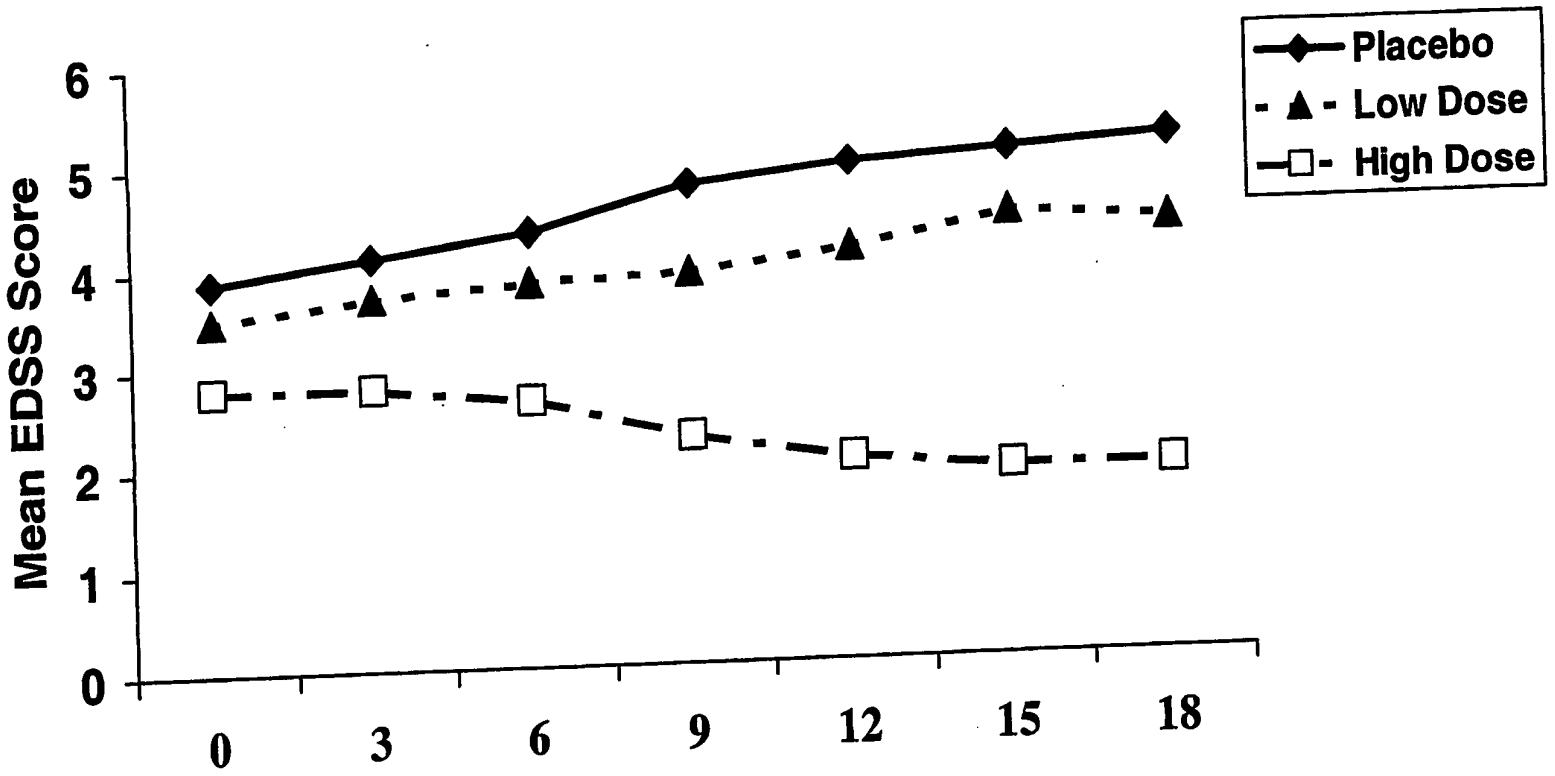

Placebo
Low Dose
High Dose
Mean EDSS Score
0
1
2
3
4
5
6
0
3
6
9
12
15
18

FIGURE3


Placebo
Low Dose
High Dose
Mean EDSS Score
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1
2
3
4
5
6
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3
6
9
12
15
18

FIGURE4

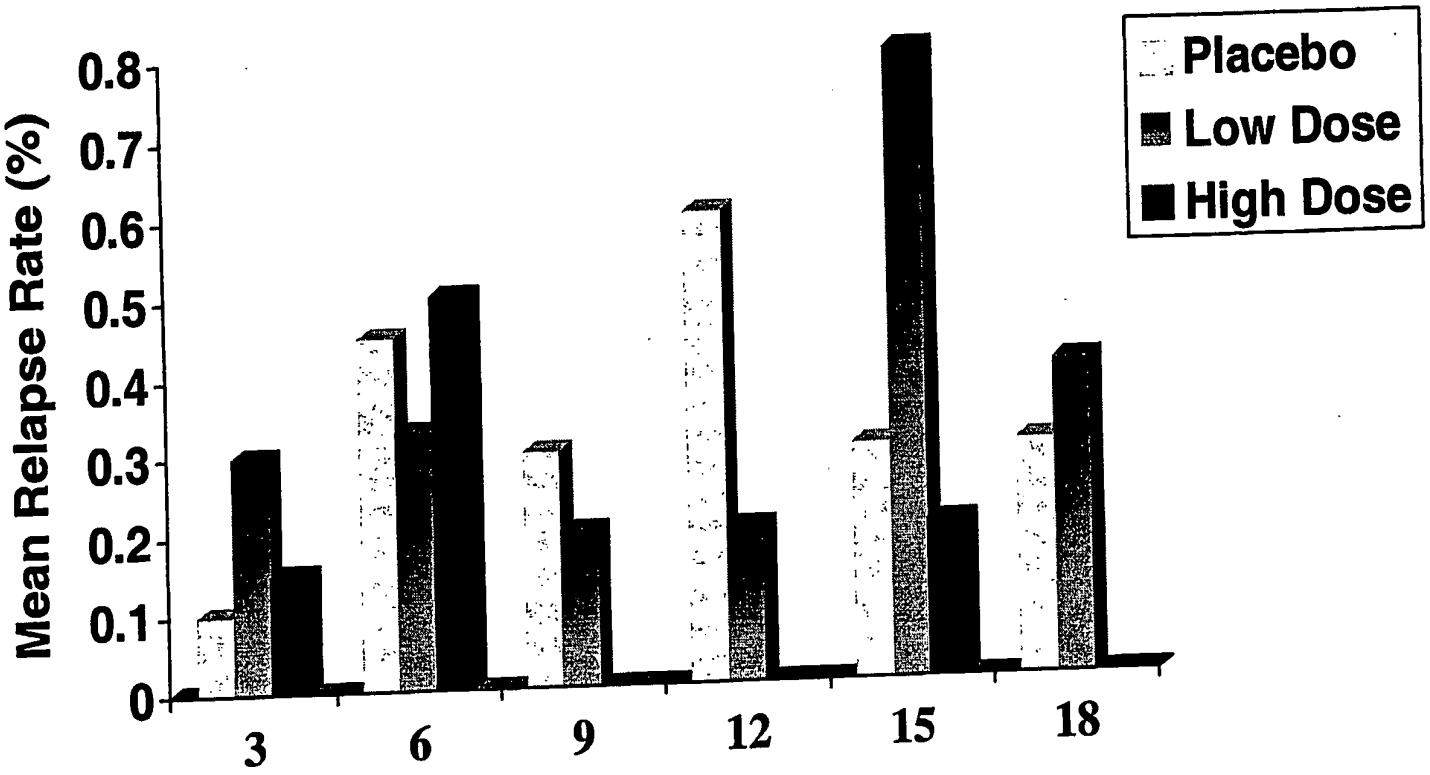
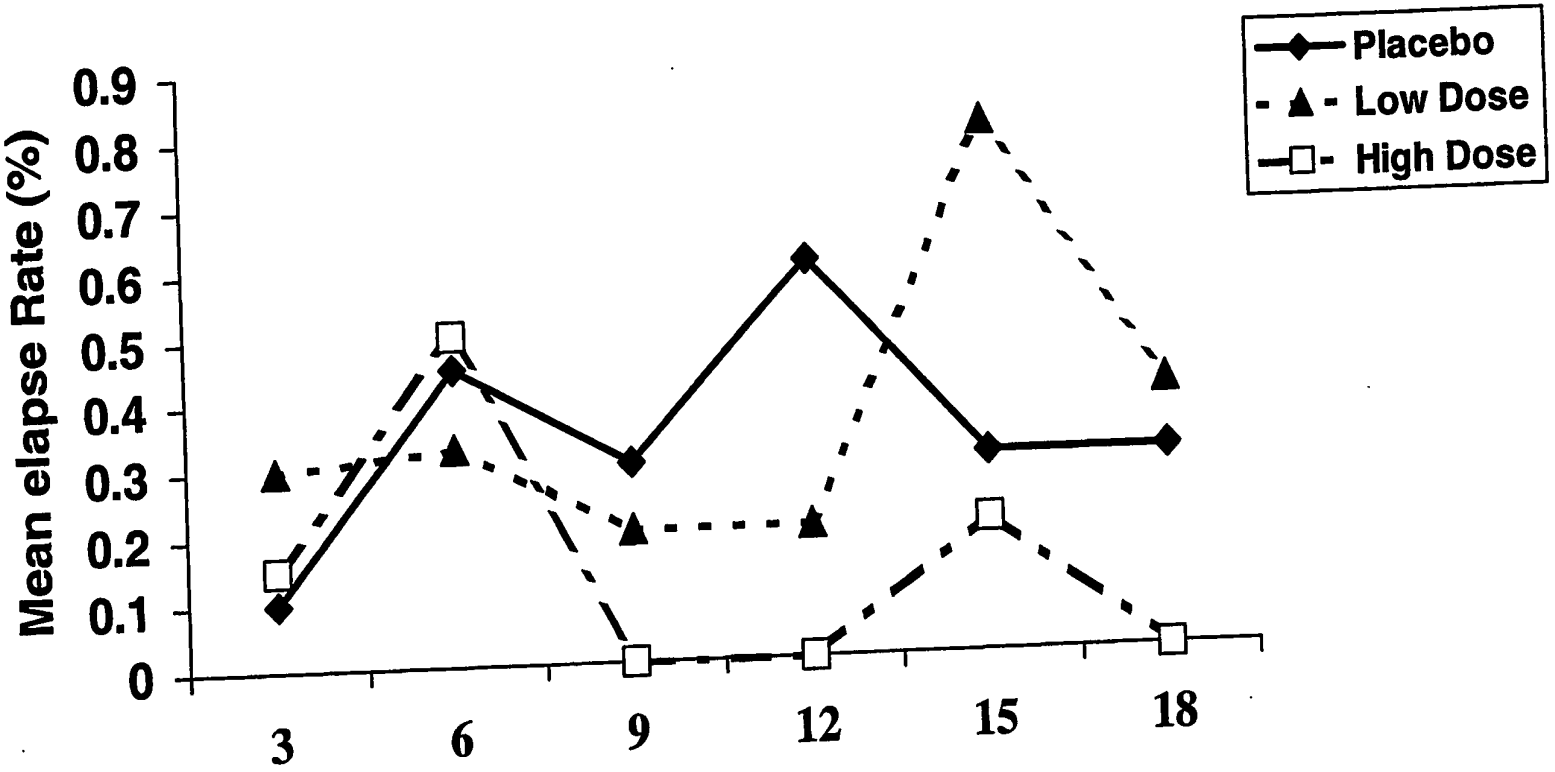

Mean Relapse Rate (%)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
3
6
9
12
15
18
Placebo
Low Dose
High Dose

FIGURE5


Placebo
Low Dose
High Dose
Mean elapse Rate (%)
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0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
3
6
9
12
15
18